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## APPENDIX A - COPY OF CLAIMS ON APPEAL 67-97

- 1 ~~67~~. An isolated molecule comprising a polypeptide that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes having a sequence that
- (a) has no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding amino acid positions in a CTL epitope which is
- LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2),
- QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO:3),
- KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28), or
- LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35), or
- (b) has no more than one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is
- ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),
- LLCPAGHAV (NS3<sub>1169-1177</sub>; SEQ ID NO:26), or
- SLMAFTAABV (NS4<sub>1789-1797</sub>; SEQ ID NO:34),
- wherein said molecule comprises at least eight amino acids and less than 50 amino acids, with the provisos that (i) when said selected CTL epitope is SLMAFTAABV (NS4<sub>1789-1797</sub>; SEQ ID NO:34), then said molecule comprises from at least eight amino acids to less than 25 amino acids, or (ii) when said selected CTL epitope is LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2) then said molecule comprises at most ten amino acids.
- 2 ~~68~~. The molecule of claim ~~67~~, wherein the isolated peptide has less than 20 amino acids.
- 3 ~~69~~. The molecule of claim ~~67~~, wherein the isolated peptide has from 8 to 12 amino acids.
- 4 ~~70~~. The molecule of claim ~~67~~, wherein the isolated peptide has 9 or 10 amino acids.

5 71. <sup>1 2 3 4</sup> The molecule of claim ~~67~~, ~~68~~, ~~69~~, or ~~70~~, wherein the isolated molecule has a sequence that has no more than a total of one amino acid substitution, deletion or insertion at the corresponding position as in LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2).

6 72. <sup>1 2 3 4</sup> The molecule of claim ~~67~~, ~~68~~, ~~69~~, or ~~70~~, wherein the isolated molecule has a sequence that has no more than a total of one amino acid substitution, deletion or insertion at the corresponding position as in QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO:3).

7 73. <sup>1 2 3 4</sup> The molecule of claim ~~67~~, ~~68~~, ~~69~~, or ~~70~~, wherein the isolated molecule has a sequence that has no more than a total of one amino acid substitution, deletion or insertion at the corresponding position as in KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28).

8 74. <sup>1 2 3 4</sup> The molecule of claim ~~67~~, ~~68~~, ~~69~~, or ~~70~~, wherein the isolated molecule has a sequence that has no more than a total of one amino acid substitution, deletion or insertion at the corresponding position as in LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35).

9 75. An immunogenic composition that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes (CTL) comprising molecule which comprises a peptide having a sequence that has no more than a total of a total of two amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),

LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2),

QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO:3),

KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28), or

LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35) or

has no more than a total of one substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

LLCPAGHAV (NS3<sub>1169-1177</sub>; SEQ ID NO:26),

SLMAFTAAV (NS4<sub>1789-1797</sub>; SEQ ID NO:34), or

ILDSFDPLV (NS5<sub>2252-2260</sub>; SEQ ID NO:42).

- 10 ~~76~~. The immunogenic composition of claim ~~75~~<sup>9</sup>, wherein the immunogenic composition further comprises a label selected from the group consisting of a radioactive label, an enzymatic label, and a fluorescent label.
- 11 ~~77~~. The immunogenic composition of claim ~~76~~<sup>9</sup>, wherein the immunogenic composition further comprises a solid matrix.
- 12 ~~78~~. The immunogenic composition of claim ~~76~~<sup>9</sup>, wherein the immunogenic composition further comprises a carrier molecule.
- 13 ~~79~~. The immunogenic composition of claim ~~76~~<sup>9</sup>, wherein the carrier molecule comprises a protein or an immunogenic lipid.
- 14 ~~80~~. The immunogenic composition of claim ~~76~~<sup>9</sup>, wherein the immunogenic composition further comprises a T-helper lymphocyte epitope.
- 15 ~~81~~. The immunogenic composition of claim ~~76~~<sup>9</sup>, wherein the immunogenic composition further comprises an additional peptide.
- 16 ~~82~~. The immunogenic composition of claim ~~81~~<sup>15</sup>, wherein the additional peptide has a sequence that has no more than a total of two amino acid substitutions, deletions or insertions at the corresponding positions as in KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28).
- 17 ~~83~~. A method of stimulating a cytotoxic T-lymphocyte (CTL) response to an hepatitis C viral immunogen, comprising contacting an HLA class I-restricted cytotoxic T lymphocyte with a composition comprising a peptide that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes comprising a sequence that has no more than a total of two single

amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),  
LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2),  
QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO: 3),  
KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28), or  
LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35) or

has no more than a total of one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),  
LLCPAGHAV (NS3<sub>1169-1177</sub>; SEQ ID NO:26),  
SLMAFTAAV (NS4<sub>1789-1797</sub>; SEQ ID NO:34), or  
ILDSFDPLV (NS5<sub>2252-2260</sub>; SEQ ID NO:42).

<sup>17</sup>  
13 ~~84~~. The method of claim ~~83~~<sup>17</sup>, wherein the contacting occurs in a mammal.

<sup>17</sup>  
14 ~~85~~. The method of claim ~~83~~<sup>17</sup>, wherein the mammal is free of HCV disease, is a carrier of HCV, or is afflicted with HCV disease.

<sup>17</sup>  
20 ~~86~~. The method of claim ~~83~~<sup>17</sup>, wherein the contacting occurs *in vitro*.

<sup>17</sup>  
21 ~~87~~. The method of claim ~~83~~<sup>17</sup>, wherein the peptide comprises the sequence which is ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1).

22 ~~88~~. A method of detecting cytotoxic T cells that respond to a T cell epitope of hepatitis C virus (HCV), the method comprising the steps of:

- (a) preparing HLA class I-restricted cytotoxic T cells;
- (b) preparing HLA class-I matched and -mismatched target cells;

(c) containing separately matched and mismatched target cells with a composition comprising a peptide that induces an HCV-specific response in cytotoxic T lymphocytes having the sequence that has no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),  
LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2),  
QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO: 3),  
KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28), or  
LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35) or

has no more than a total of one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

LLCPAGHAV (NS3<sub>1169-1177</sub>; SEQ ID NO:26),  
SLMAFTAAV (NS4<sub>1789-1797</sub>; SEQ ID NO:34), or  
ILDSFDPLV (NS5<sub>2252-2260</sub>; SEQ ID NO:42);

(d) combining the cytotoxic T cells separately with the matched and mismatched target cells; and

(e) measuring cytolysis.

<sup>22</sup>  
23 ~~29~~. The method of claim ~~28~~, wherein the cytotoxic T cells are combined with HLA class I-matched lymphocytes.

24 ~~30~~. A pharmaceutical composition comprising a peptide that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes having a sequence that has no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),  
LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2),  
QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO: 3),  
KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28), or

LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35) or  
 has no more than a total of one single amino acid substitution, deletion or insertion at the  
 corresponding amino acid positions as in a CTL epitope which is  
 LLCPAGHAV (NS3<sub>1169-1177</sub>; SEQ ID NO:26),  
 SLMAFTAAV (NS4<sub>1789-1797</sub>; SEQ ID NO:34), or  
 ILDSFDPLV (NS5<sub>2252-2260</sub>; SEQ ID NO:42), and  
 a pharmaceutically acceptable carrier.

24

25 91. The pharmaceutical composition of claim 90, wherein the peptide has less than 20 amino acids.

26 92. A conjugate comprising

(a) a molecule, which comprises:

a polypeptide an having no more than a total of two single amino acid  
 substitutions, deletions or insertions at the corresponding positions as in a  
 CTL epitope which is

ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),

LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2),

QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO:3),

KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28), or

LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35) or

has no more than a total of one single amino acid substitution, deletion or  
 insertion at the corresponding amino acid positions as in a CTL epitope  
 which is

LLCPAGHAV (NS3<sub>1169-1177</sub>; SEQ ID NO:26),

SLMAFTAAV (NS4<sub>1789-1797</sub>; SEQ ID NO:34), or

ILDSFDPLV (NS5<sub>2252-2260</sub>; SEQ ID NO:42),; and

(b) a substance selected from the group consisting of a radiolabel, an enzyme, a  
 fluorescent label, a solid matrix, a carrier and an additional molecule of (a).

27 <sup>26</sup>93. The conjugate of claim <sup>26</sup>92, wherein said carrier comprises an immunogenic lipid or protein.

28 <sup>26</sup>94. A conjugate of claim <sup>26</sup>92 comprising two molecules, each comprising:  
a polypeptide no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is  
ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),  
LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2),  
QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO: 3),  
KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28), or  
LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35) or  
has no more than a total of one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is  
LLCPAGHAV (NS3<sub>1169-1177</sub>; SEQ ID NO:26),  
SLMAFTAAV (NS4<sub>1789-1797</sub>; SEQ ID NO:34), or  
ILDSFDPLV (NS5<sub>2252-2260</sub>; SEQ ID NO:42).

29 <sup>28</sup>95. The conjugate of claim <sup>28</sup>94, wherein at least one of said molecules comprises at least eight amino acids and less than 50 amino acids.

30 <sup>28</sup>96. The conjugate of claim <sup>28</sup>94, further comprising a T helper epitope.

31 <sup>28</sup>97. An isolated molecule comprising a polypeptide that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes having a sequence that has  
(a) no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding amino acid positions in a CTL epitope which is  
LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2),  
QLRRHIDLLV (E1<sub>257-266</sub>; SEQ ID NO:3),

KLVALGINAV (NS3<sub>1406-1415</sub>; SEQ ID NO:28), or

LLFNILGGWV (NS4<sub>1807-1816</sub>; SEQ ID NO:35), or

(b) has no more than one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

ADLMGYIPLV (Core<sub>131-140</sub>; SEQ ID NO:1),

LLCPAGHAV (NS3<sub>1169-1177</sub>; SEQ ID NO:26), or

SLMAFTAAV (NS4<sub>1789-1797</sub>; SEQ ID NO:34),

wherein said polypeptide comprises at least eight amino acids and less than 50 amino acids, wherein said selected CTL epitope maintains an

XaaLeuXaaXaaXaaXaaXaaXaaVal or

XaaLeuXaaXaaXaaXaaXaaXaaVal motif,

with the provisos that (a) when said selected CTL epitope is SLMAFTAAV (NS4<sub>1789-1797</sub>; SEQ ID NO:34), then said polypeptide comprises from at least eight amino acids to less than 25 amino acids, and (b) when said selected CTL epitope is LLALLSCLTV (Core<sub>178-187</sub>; SEQ ID NO:2) then said molecule comprises at most ten amino acids.

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